

Eradication of Methicillin-Resistant *Staphylococcus aureus* and of *Enterobacteriaceae* Expressing Extended-Spectrum Beta-Lactamases on a Model Pig Farm

Ricarda Maria Schmuthausen,^{a,b} Sophia Ricarda Kellner,^b Sophia Veronika Schulze-Geisthoevel,^b Sylvia Hack,^c Steffen Engelhart,^c Isabel Bodenstern,^a Nahed Al-Sabti,^a Marion Reif,^a Rolf Fimmers,^d Barbara Körber-Irrgang,^e Jürgen Harlizius,^f Achim Hoerauf,^a Martin Exner,^c Gabriele Bierbaum,^a Brigitte Petersen,^b Isabelle Bekerredjian-Ding^{a,g}

Institute of Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Bonn, Germany^a; Institute of Animal Science, Preventive Health Management Group, University of Bonn, Bonn, Germany^b; Institute for Hygiene and Public Health, University Hospital Bonn, Bonn, Germany^c; Institute of Medical Biometry, Epidemiology and Computer Science, University Hospital Bonn, Bonn, Germany^d; Antiinfectives Intelligence GmbH, Campus University of Applied Sciences Bonn-Rhein-Sieg, Rheinbach, Germany^e; Chamber of Agriculture of North Rhine-Westphalia, Bonn, Germany^f; Division of Microbiology, Paul-Ehrlich-Institut, Langen, Germany^g

Colonization of livestock with bacteria resistant to antibiotics is considered a risk for the entry of drug-resistant pathogens into the food chain. For this reason, there is a need for novel concepts to address the eradication of drug-resistant commensals on farms. In the present report, we evaluated the decontamination measures taken on a farm contaminated with methicillin-resistant *Staphylococcus aureus* (MRSA) and *Enterobacteriaceae* expressing extended-spectrum β -lactamases (ESBL-E). The decontamination process preceded the conversion from piglet breeding to gilt production. Microbiological surveillance showed that the decontamination measures eliminated the MRSA and ESBL-E strains that were detected on the farm before the complete removal of pigs, cleaning and disinfection of the stable, and construction of an additional stable meeting high-quality standards. After pig production was restarted, ESBL-E remained undetectable over 12 months, but MRSA was recovered from pigs and the environment within the first 2 days. However, *spa* (*Staphylococcus aureus* protein A gene) typing revealed acquisition of an MRSA strain (type t034) that had not been detected before decontamination. Interestingly, we observed that a farmworker who had been colonized with the prior MRSA strain (t2011) acquired the new strain (t034) after 2 months. In summary, this report demonstrates that decontamination protocols similar to those used here can lead to successful elimination of contaminating MRSA and ESBL-E in pigs and the stable environment. Nevertheless, decontamination protocols do not prevent the acquisition of new MRSA strains.

Numerous studies have highlighted the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) (1–4) and *Enterobacteriaceae* expressing extended-spectrum β -lactamases (ESBL-E) (5–7) in livestock production, particularly in pigs. Hence, there is an ongoing debate whether the use of antibiotics in food animal production represents an important source of continuous spread of MRSA and ESBL-E to humans (8–10). Farmers are confronted with two different consequences of this problem: the potential danger of animal colonization with drug-resistant bacteria for (i) humans living on or in the vicinity of farms (11–13) and for (ii) consumers of animal products (14–18). Indeed, livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) have been found in humans living and working in close contact with pigs and also in hospitals in rural areas (11, 19–25). In contrast, ESBL-E could possibly be transferred from animals to humans via meat products (16, 26–28). As a consequence, control points to limit transmission of resistant pathogens “from stable to table” have been demanded (29).

Notably, bacteria not only persist on/in the living animal but also on surfaces that are in contact with the animals, such as barn walls and equipment (30, 31). LA-MRSA isolates were detected in dust samples from the investigated breeding farms in Germany as part of the EFSA (European Food Safety Authority) study, which focuses on objectives such as antimicrobial resistance, foodborne zoonotic diseases, and monitoring of feed- and foodborne diseases (32). Friese et al. (33) have shown the occurrence of LA-MRSA in the stable air of a fattening farm, while ESBL-expressing

Escherichia coli isolates have been isolated from sewers surrounding livestock production sites (34). Furthermore, in the farm environment both commensal and environmental bacteria serve as reservoirs for the transfer of antimicrobial resistance genes to pathogenic bacteria (35–37), thus complicating the disease course and therapeutic regimens.

Consequently, assessment points in pig housing conditions, environmental care, animal health, and food product safety and quality, as well as consumer acceptance have already been defined, and critical control points (CCP [based on the “hazard analysis and critical control points” concept]) that require the attention of

Received 8 June 2015 Accepted 18 August 2015

Accepted manuscript posted online 4 September 2015

Citation Schmuthausen RM, Kellner SR, Schulze-Geisthoevel SV, Hack S, Engelhart S, Bodenstern I, Al-Sabti N, Reif M, Fimmers R, Körber-Irrgang B, Harlizius J, Hoerauf A, Exner M, Bierbaum G, Petersen B, Bekerredjian-Ding I. 2015. Eradication of methicillin-resistant *Staphylococcus aureus* and of *Enterobacteriaceae* expressing extended-spectrum beta-lactamases on a model pig farm. *Appl Environ Microbiol* 81:7633–7643. doi:10.1128/AEM.01713-15.

Editor: H. L. Drake

Address correspondence to Ricarda Maria Schmuthausen, ricarda.schmuthausen@ukb.uni-bonn.de, or Isabelle Bekerredjian-Ding, isabelle.bekerredjian-ding@pei.de.

G.B., B.P., and I.B.-D. contributed equally to this article.

Copyright © 2015, American Society for Microbiology. All Rights Reserved.

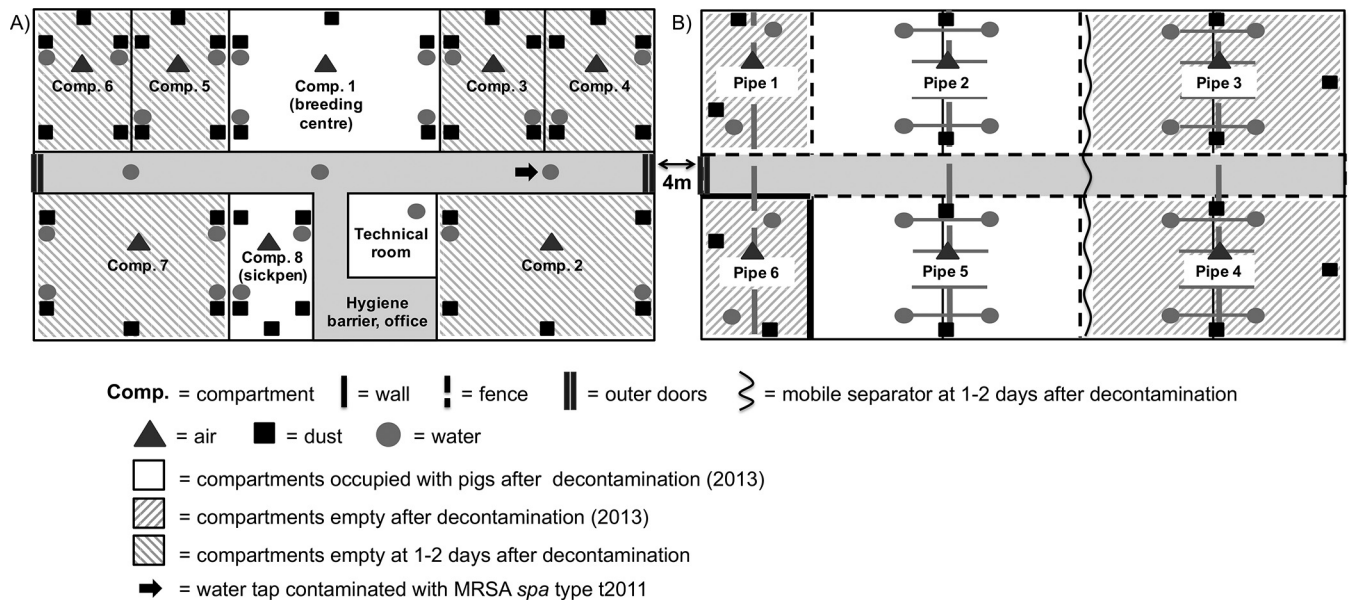


FIG 1 Sampling plan of environmental samples before and after culling and decontamination in the old (A) and new (B) stables.

the food processing industry (38) have been implemented in quality control systems (29). However, the strict implementation of infection control measures to reduce the prevalence of resistant bacteria is challenging in pig production (39, 40). Hunter et al. (8) doubt that strategies established in human medicine could be successfully modified for use in livestock. Due to the high number of pigs in a confined space, individual decolonization as conducted on human patients is nearly impossible; moreover, the contaminated environment in the stable needs to be cleaned and disinfected (41).

To date, the efficacy of disinfection measures on farms contaminated with drug-resistant bacteria is under debate. Our report summarizes the results of microbiological surveillance following an attempt to eradicate (completely eliminate) MRSA and ESBL-E from a model pig production farm. Extensive hygiene measures were employed to fight colonization in piglets, sows, and gilts, as well as to decontaminate the stable environment.

MATERIALS AND METHODS

Formal surveillance procedures and ethics statement. This report is an observational quality control and a follow-up monitoring of a former hygiene monitoring program. The model pig farm previously participated in a hygiene monitoring program supported by their pig producer association in collaboration with the agricultural faculty of the University of Bonn (carried out in 2012 to 2013) and was found to be contaminated with MRSA and ESBL-E. The farmer (owner) approached the authors of this article for follow-up surveillance within the framework of the routine hygiene monitoring program (2013 to 2014). He agreed with the collection of air and other environmental samples and the sampling of the pigs on the farms. Hence, these samples were taken during routine sampling for monitoring, and the sampling itself was noninvasive. The data obtained during this routine monitoring were analyzed retrospectively. The farmer provided information on the antibiotic classes administered to his pigs. This information was verified in his livestock protocol. The results of this report were communicated to the farmer. All data were handled anonymously.

According to German animal welfare legislation, this report is not an animal experiment. An approval by the regulatory body or an animal

welfare committee is not necessary. Nevertheless, all measures taken strictly follow the terms set by the animal welfare committee of the University of Bonn. With regard to the human samples, no personal data were used or stored for this observational report. The farm owner and his farm personnel were informed about the follow-up report and participated on a voluntary basis. In accordance with the declaration of Helsinki/Seoul, written informed consent is available from all human subjects involved. The ethics committee of the Medical Faculty of the University of Bonn was involved and approved the procedures and the publication of the results (reference no. 226/15).

Time course and sampling approach. The report summarizes the results obtained during microbiological surveillance over a period of one and a half years (from June 2012 to February 2014): (i) baseline values obtained during a routine monitoring program in 2012, (ii) surveillance before and after decontamination (process of cleaning and disinfection) in 2013, and (iii) follow-up monitoring in 2014 (i.e., 1 year after decontamination).

The decontamination was performed during March and April 2013 and carried out by a state-certified disinfectant from Destec (Rees, Germany). All pigs were culled (i.e., underwent precautionary killing of animals to prevent the spread of animal diseases).

In the old stable (A), the individual compartments were completely separated and could only be accessed through the central corridor (Fig. 1). Furthermore, a new stable (B) had been added, which was built in series with the old stable but had to be entered separately. The new stable contained five gilt compartments (waiting areas 1 to 5) in an inside open-housing system (compartments were separated solely by low barriers). Only quarantine compartment 6 was totally separated.

Repopulation of the new stable (B) with pigs was conducted in two steps: within the first 2 days, 250 gilts arrived, and in the first month, 320 gilts arrived. The new incoming gilts were stabled according to their transport groupings, which also corresponded to their age groups. This means that these gilts stayed together and were not mixed. Beyond this, no animal movements occurred. However, the gilts were inseminated in the breeding center (compartment 1 [comp. 1]) in the old stable (A) and returned to their waiting stable (B).

Microbiological surveillance consisted of screening for MRSA and ESBL-E in pigs (sows and gilts) and air in 2012 (9 months before decontamination), pigs, air, water, and dust in 2013 in the old stable 1 to 2 days

before decontamination, and once a month for 3 months (1 to 2 days, 1 month, and 2 months) after decontamination in both the old (A) and new (B) stables and in pigs in 2014 (12 months after). In the first 2 months after decontamination, only two compartments (compartment 1, a breeding center for gilts, and compartment 8, a sick pen) in the old stable were populated with pigs (Fig. 1). However, 12 months after decontamination the old stable was almost fully occupied. For the open parts of the stable, the sampling plan was oriented following the water pipes of the compartments (with one pipe defined as one compartment) (Fig. 1). The restabbling with pigs occurred as follows. Directly after the new construction, compartments 3, 4, and 6 were temporarily separated by foil and were free of pigs. One, 2, and 12 months later, all compartments were filled with gilts. Dust samples and water samples were taken in all four quadrants of a compartment.

Cleaning and disinfection. The decontamination process was devised by Destec (Reed, Germany) and comprised two parts: (i) cleaning and (ii) disinfection. Cleaning was performed by the farmer and the farm personnel. Before cleaning, all technical installations (in farrowing pens), the ventilation and air conditioning system, and water and feeding pipes as well as the ground floor were dismantled and disposed of. New technical equipment and pipes were reinstalled after disinfection. After high-pressure cleaning and foaming, the stable was purified with Alkatens (potassium hydroxide solution, amphoteric surfactants, and complexing agents) (EWABO Chemikalien GmbH and Co., KG, Wietmarschen, Germany). The disinfection procedure involved (i) wet surface disinfection with Aldekol DES 03 (EWABO Chemikalien GmbH and Co., KG, Wietmarschen, Germany) [glutaraldehyde, formaldehyde, benzyl-(C₁₂ to C₁₆)-alkyl dimethyl ammonium chloride], which was performed by Destec over a period of 2 days, and (ii) hot nebulization (1,000°C, <10-μm drop diameter) for the manure pit, grooves, ventilation system, and feeding installation for 48 h. After decontamination and during the whole study period, access to the stable was only permitted via a hygiene barrier, which included showering and a complete change of clothes. Additionally, entry was restricted for persons who had had contact with pigs in the preceding 48 h.

Sample collection and laboratory analyses. All collected samples were transported (cooled to 4°C) to the laboratory for further processing within 4 h. Amies medium and charcoal swabs were supplied by Mast Diagnostica GmbH (Reinfeld, Germany). All swabs were streaked onto Columbia–5% sheep blood agar plates and selective CHROMagar MRSA (nasal swabs) or ESB_L-Screening CHROMagar ESB_L (anal swabs) (Mast Diagnostica GmbH, Reinfeld, Germany). Plates were incubated at 37 ± 1°C in 5% CO₂ for 48 h, with a first reading after 24 h. Pink colonies (at least one) were streaked on Columbia–5% sheep blood agar plates. Quantitative counting of CFU was not performed for either nasal or anal swabs.

Sample collection in pigs. During routine monitoring in 2012 a total of 20 pigs at two different age groups (10 suckling piglets, 10 sows) were randomly tested. In 2013 before the decontamination, 30 sows of the former monitored population in the old stable were sampled. Sows were chosen randomly from all compartments. After decontamination, sows were housed in the old stable and gilts were housed in the new stable. There were always considerably fewer sows than gilts. One to 2 days after the arrival of the first lot of new pigs (about 250), samples were obtained from about 10% of the pigs from each stable (8 sows housed in compartments 1 and 8 of the old stable and 22 gilts in compartments 2 and 5 in the new stable) in a random approach. In the following months, the number of pigs increased by 320. At any time point, when new pigs entered the new stable prophylactic doxycycline was orally administered via fodder, and samples were obtained. Due to the increasing number of gilts in the new stable, the number of samples from this stable also increased to 52 (or 53) in compartments 1 to 6 (ranging from 5 to 10 pigs per compartment, depending on the occupancy rate) (Fig. 1). During follow-up monitoring in 2014, a total of 38 gilts were sampled, 19 from each (old and new) stable. The sampling strategy referred to compartments and therefore was de-

signed in such a way that pigs from all compartments were sampled; in consequence, it could not be ensured that individual pigs were resampled. Sampling followed the same procedure during all parts of the report. Nasal swabs (inserted 4 cm into both anterior nares along the nasal septum) were collected for MRSA detection and intrarectal swabs for ESB_L-E-screening.

Human and companion animal specimens. We obtained samples from all humans living and working on the farm, as well as from the nares of companion animals (two dogs and two horses [42, 43]). The farmer and farm personnel contributed nasal samples from the vestibule of both anterior nares.

From humans and from companion animals, only nasal swabs were collected for MRSA detection. No ESB_L-E screening was performed.

Decolonization of personnel was performed with the MRE hygiene set (B. Braun Melsungen AG, Melsungen, Germany).

Air samples. During air sample collection, doors were closed. Air was collected in the center (40 cm in height) of all compartments (empty and utilized compartments) (Fig. 1) by impaction employing the MAS-100 NT (Merck KGaA, Darmstadt, Germany). During this procedure, the air was drawn through a perforated lid (300 holes, each with a 0.6-mm diameter), and the accelerated particles impacted the surface of selective growth media (CHROMagar MRSA and CHROMagar ESB_L; Mast Diagnostica GmbH, Reinfeld, Germany). MRSA was detected in an air volume of 50 liters (at a flow rate of 100 liters/min for 30 s). Two measurements were needed for the screening of ESB_L-E (air volume of 500 liters at a flow rate of 100 liters/min for 5 min per compartment). The air samples were taken consecutively with time intervals of 30 to 60 s. After each measurement, the system was disinfected with alcohol pads (B. Braun Melsungen AG, Melsungen, Germany). Colonies were counted as total number of CFU per cubic meter and specified according to statistical correction of Feller with the species-specific correction factor P_i/r , where P_i is the probable statistical total and r is the number of CFU counted on a standard petri dish (44). The minimum detection limit was 8 CFU/m³.

Dust samples. Dust was sampled in both animal houses at five (old stable) and four (new stable) sample points per compartment in front and rear positions (Fig. 1). Dust samples were obtained from (i) an area of 10 by 10 cm (height, 1.5 m) on a flat surface of the window and/or door sills and (ii) an area of 2 by 50 cm (height, 1.0 m) from bay separations. During sampling, doors were kept closed. The dust was collected into sterile 100-ml polystyrene containers (no. 225170; Greiner, Frickenhausen, Germany) using sterile cotton swabs (Mastaswab MD 514; Mast Diagnostica, Reinfeld, Germany). After precise weighing, dust samples were suspended in 10 ml sterile NaCl (0.9%) and shaken for 5 min. This suspension was then diluted in three steps (1:10, 1:100, and 1:1,000) in sterile NaCl before 100 μl of the suspension and each dilution was plated on CHROMagar MRSA and CHROMagar ESB_L (Mast Diagnostica, Reinfeld, Germany). Results were calculated as CFU/100 cm².

Water samples. Water samples were collected by a research assistant with valid admission for water sampling according to the Drinking Water Regulation 2001 (45).

The water supply of the farmer's house and the entire stable complex is managed via a central water pumping station in the service area of the old stable. The central pump transports the water up from the farm's wells. The drinking water is controlled regularly. The main pipeline system is installed in the central corridors of both the old and the new stables and connects them in series. The constructions of the water network are very similar in both stables. From the connecting main pipeline, large pipes branch off into the respective compartments and branch again along the walls/separations.

In the old stable, small branch-offs supply water to each nipple drinker (with no drip up) per sow pen. Compartments 2 to 7 contain 14 sow pens (one per sow). Compartment 1 has almost 80 nipple drinkers that protrude from the long water pipes at animal height.

In the new open-plan stable, water pipes run along the feeding pipes. Four small branch-offs per compartment supply water to nipple drinkers

(with no drip up). No water flow occurred prior to sampling of the nipple drinkers. At the most peripheral sampling points of the water system, an additional sample was taken after a water flow of 3 min. Samples of the main water system were obtained from three sites in the central aisle of the old stable and from the central pump: water taps in the central corridor were flamed, and water collection was carried out after 30 s of water flow. Water splashing and overflowing were avoided. Animals had no contact with the main line taps. Within the compartments of the old stable, water was obtained from the (i) right and/or (ii) left branches of the compartment pipe (compartments 2 to 7) and nipple drinkers (compartment 1), respectively. If possible, one water sample was sampled at the peripheral end, and the other sample was collected at the beginning of the compartment branch-off. The water in the new stable was collected from the first and last nipple drinker of the compartment pipe. Here, pig contact was possible.

Approximately 100 ml of water was collected in a sterile polystyrene cup (article no. 225170; Greiner, Frickenhausen, Germany) and filtered twice (2×50 ml) through a sterile nitrocellulose membrane filter (pore size, $0.45 \pm 0.02 \mu\text{m}$) (Millipore) according to Schulz and Hartung (46). After filtration, the membrane was placed on selective CHROMagar MRSA and CHROMagar ESBL agar plates (Mast Diagnostica, Reinfeld, Germany). In addition, three 10-fold dilutions were prepared using sterile NaCl, and 100 μl of each dilution was plated on CHROMagar MRSA and CHROMagar ESBL.

Liquid manure and fodder samples. Liquid manure and fodder were sampled only 1 to 2 days before the decontamination procedure. Liquid manure was sampled from the manure pits beyond the old stable. Fodder samples were collected while filling the feed troughs. Both samples were transported in sterile 100-ml polystyrene cups (no. 225170; Greiner, Frickenhausen, Germany).

After mixing, 10 g of each sample was transferred into 90 ml sterile NaCl (0.9%) and homogenized using a Stomacher blender (260 rpm for 2 min) (Stomacher 400 Circulator; Seward United Kingdom, West Sussex, United Kingdom). This suspension was further diluted to 10^{-4} . From each dilution, 100 μl was plated onto CHROMagar ESBL and CHROMagar MRSA. Typical colonies were counted, and *S. aureus* colonies were confirmed by a plasma coagulase test and matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Vitek MS, bioMérieux SA, Marcy l’Etoile, France).

Confirmation of MRSA and *spa* typing. Species identification of *S. aureus* was confirmed by coagulase testing and MALDI-TOF MS (Vitek MS, bioMérieux SA, Marcy l’Etoile, France). Antimicrobial resistance was determined by Vitek-2 (bioMérieux SA, Nuertingen, Germany) employing the card AST 632 (where AST represents “automatic susceptibility typing”) (47). Breakpoints for classification of resistance and susceptibility were determined based on international standards (http://www.eucast.org/clinical_breakpoints/). Selected strains from pigs and environmental samples and all human MRSA isolates were *spa* typed as described by Harmsen et al. (48) and allocated to *spa* types using the *spa* typing website (<http://www.spaserver.ridom.de/>).

Identification, antimicrobial susceptibility testing, and molecular typing of ESBL-E. ESBL-E was identified by MALDI-TOF MS (bioMérieux SA, Nuertingen, Germany) using Axima@SARAMIS (bioMérieux SA, Nuertingen, Germany). Antimicrobial susceptibility testing of *E. coli* isolates was performed on a Vitek-2 employing the cards AST 214 and AST 248 (bioMérieux SA, Nuertingen, Germany). Breakpoints for classification of resistance and susceptibility were determined based on EUCAST (http://www.eucast.org/clinical_breakpoints/). Human clinical breakpoints were primarily used to investigate resistance against antimicrobials used in human medicine.

ESBL was further confirmed by PCR detection of CTX-M (AID Diagnostika GmbH, Straßberg, Germany).

Statistical analysis. For the comparison of colonization rates of pigs (9 months and 1 to 2 days prior to culling and decontamination versus 12 months and 1 to 2 days after culling and decontamination), alternating

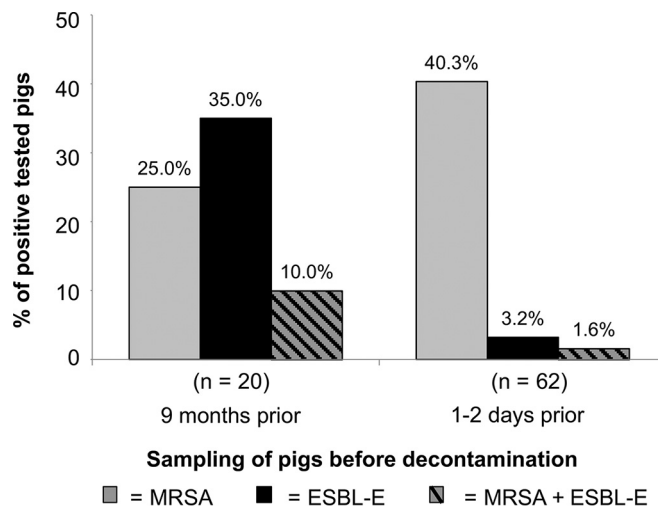


FIG 2 Prevalence of MRSA and ESBL-E in pigs before culling and decontamination. Shown are the percentages of pigs colonized with MRSA and/or ESBL-E before decontamination ($n = 82$).

logistic regression was used to take into account possible dependencies between measurements at the same time in the same compartment.

RESULTS

Situation at the outset. (i) Prevalence of MRSA and ESBL-E in pigs. This article summarizes the observations made on an individual pig farm in North-Rhine-Westphalia (Germany). The farm had participated in 2012 in a routine hygiene monitoring program, which was coordinated by the Faculty of Agriculture of the University of Bonn. Due to high levels of MRSA and ESBL-E colonization (Fig. 2), the farmer had decided to perform a professional decontamination. The farmer’s plan included (i) complete culling of the original pig population, (ii) decontamination of the existing stable (old stable) with cleaning and disinfection, and (iii) decolonization of an employee continuously colonized with MRSA (staff 1). The farmer then planned and performed the conversion from piglet breeding to gilt production, which included the construction of an additional gilt stable (new stable) next to the old stable (Fig. 1).

Figure 2 shows the situation at the outset: initial monitoring 9 months before decontamination revealed a high prevalence of MRSA and ESBL-E and simultaneous carriage of MRSA and ESBL-E in 10% of the piglets ($n = 20$) (Fig. 2). A second sampling 9 months later (1 to 2 days before decontamination) was performed to confirm that pigs were contaminated with either MRSA or ESBL-E ($n = 62$); one pig carried both. MRSA carriage rates of the pigs continued to be high prior to decontamination, while ESBL-E detection rates were decreased (Fig. 2). All ESBL-E isolates in this study were resistant to β -lactams, quinolones, doxycycline, tetracycline, and trimethoprim-sulfamethoxazole. The presence of ESBL-E genes was confirmed by PCR in two strains, both of which were CTX-M positive.

(ii) MRSA and ESBL-E in environmental samples. In contaminated stables, MRSA and ESBL-E have been detected in water (49, 50), air (33, 51), and dust (52, 53), as well as in fodder (33, 50) and liquid manure (33, 51). During the previous monitoring program, microbiological analysis revealed the presence of MRSA in all samples of the stable air of the model pig farm and ESBL-E had

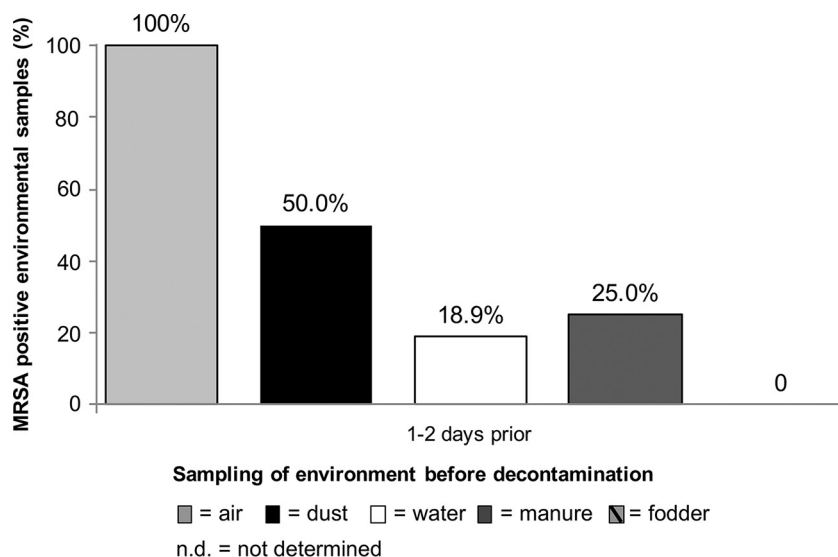


FIG 3 MRSA prevalence in environmental samples on the model pig farm. Samples from air were obtained 9 months ($n = 2$) and 1 to 2 days ($n = 8$) before culling and decontamination of the old stable. Samples from dust ($n = 22$), water ($n = 37$), manure ($n = 4$), and fodder ($n = 10$) were collected.

been detected in one out of two air samples collected within the stable. Resampling 9 months later again provided evidence for the presence of MRSA in all of the air (Fig. 3), dust, water, and manure samples collected.

In contrast, the 10 pooled fodder samples that were collected from the pig troughs were negative for both MRSA (Fig. 3) and ESBL-expressing *E. coli*. Two samples were positive for *Citrobacter amalonaticus* and one for *Kluyvera cryocrescens*. Since these species were not recovered from pigs, they were not subjected to further analyses. Four liquid manure samples were obtained, which had detection rates of 25% (1/4) for MRSA (Fig. 3) and 50% (2/4) for ESBL-E. Whenever dust tested positive in one compartment, at least one water sample was found to be positive as well, but not vice versa.

In contrast, there was no evidence of ESBL-E in the environmental samples.

After decontamination. (i) Prevalence of MRSA and ESBL-E in pigs. Before repopulation, 10% of the newly purchased sows and gilts were screened for MRSA and ESBL-E and were shown to be negative. Furthermore, native dust samples from the supplier

were confirmed by an external laboratory to be negative for MRSA and ESBL-E. Restabling with pigs occurred 1 to 2 days after the final decontamination measures or 6 weeks after construction of the new stable was finished.

Despite this, an initial screen for MRSA and ESBL-E 2 days after the arrival of the new pigs revealed a low rate of MRSA (10.0% [3/30]) but no ESBL-E. Two (out of 8) pigs (25.0%) in the old stable and one pig (out of 22) (4.5%) in the new stable were MRSA positive. In the first days, the majority of the pigs (gilts) were housed in compartments 1, 2, and 5 in the new stable, and only a few animals (sows) were stabled in compartments 1 and 8 in the old stable.

Resampling after 1 and 2 months revealed increased carriage of MRSA in pigs in both stables. An MRSA frequency of 37.5% (3/8) was found for pigs (Fig. 4) that occupied two compartments in the old stable (Fig. 1). This rate was approximately equal to the average rate of 32.7% for MRSA-positive pigs in the initial sampling before the decontamination measures. Within this sampling period, the procedure of pig restabling was completed. There was no evidence for ESBL-E carriage.

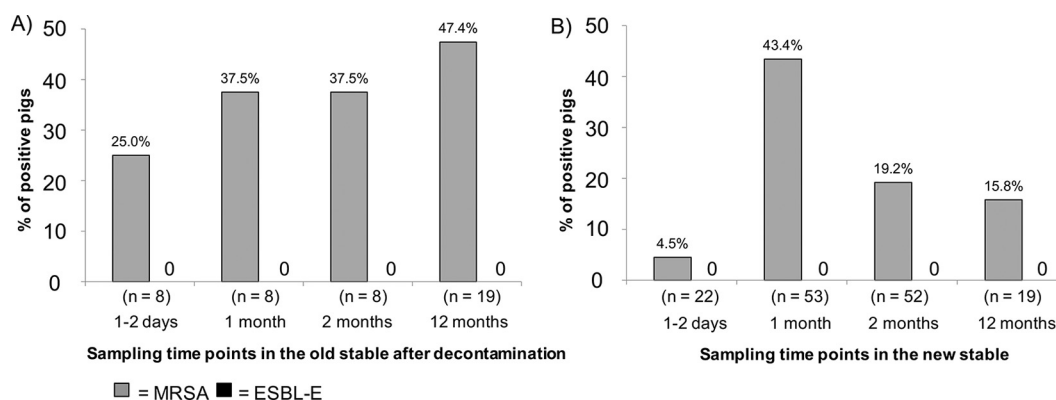


FIG 4 Percentage of pigs positive for MRSA or ESBL-E after culling and decontamination and the time points of sampling in the old (A) and new (B) stable sites.

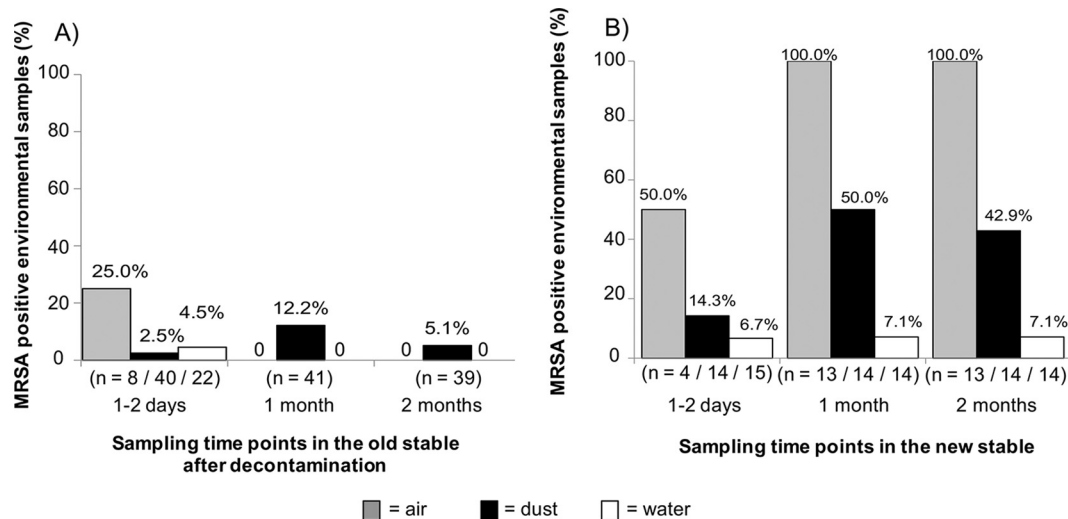


FIG 5 Percentage of environmental samples positive for MRSA after culling and decontamination and the time points of sampling in the old (A) and new (B) stable sites.

To monitor long-term effects of the decontamination procedure, we reassessed MRSA and ESBL-E carriage in pigs on the same farm after 12 months (Fig. 4). No pig was identified as positive for ESBL-E. In total (including pigs from the new and old stables), 12 out of 38 (31.6%) pigs were colonized with MRSA. This roughly corresponds to the average (32.7%) MRSA carriage in the first two samplings preceding the intervention measures (Fig. 4). The statistical analysis of the MRSA colonization of the pigs sampled at different time points 9 months and 1 to 2 days before versus 1 to 2 days and 12 months after culling and decontamination yielded a *P* value of 0.0721.

(ii) Prevalence of MRSA and ESBL-E in the environment. Sampling of pigs was accompanied by environmental sampling following the sampling scheme in Fig. 1. MRSA was detected after 24 to 48 h after the first new sows and gilts had arrived. MRSA isolates were present in samples of air, dust, and water obtained from both the old and new stables (Fig. 5).

In months 1 and 2 after the decontamination procedure, all air samples obtained in the old stable were negative, whereas all air samples collected in the new open-plan stable were positive. More generally, in the old stable only dust samples from the two pig-housing compartments (Fig. 1) were MRSA positive. In contrast, within the new stable MRSA was detected in all environmental media (air, dust, and water) (Fig. 5). In particular, MRSA detection in air correlated with that in dust. Furthermore, dust and water samples taken from one compartment seemed to correlate.

Before the farm conversion, MRSA had been detected in almost every compartment of the old stable. After the decontamination procedure, only compartments already populated by pigs were MRSA positive. In the new stable, MRSA was also present in compartments that housed pigs. One exception, however, was an air sample collected in a temporarily foil-separated and pig-free compartment (directly after conversion) that tested positive for MRSA. On the other hand, not all environmental samples in compartments with pigs were MRSA positive.

ESBL-E was not detectable in air, dust, or water samples within the first 2 months.

***spa* typing of MRSA strains.** All MRSA isolates in this study

showed resistance to all tested β -lactams, erythromycin, clindamycin, and doxycycline only, and no changes antibiotic susceptibility were observed over time. To confirm or exclude the reappearance of the MRSA strains that had been identified in the farm environment before the decontamination measures, we subjected selected MRSA isolates to *spa* typing. In total, 78 MRSA isolates were analyzed via *spa* typing, including 35 pig and 11 human isolates, as well as strains from 16 air, 13 dust, and 4 water samples. All *spa* types belonged to clonal complex 398: that is t011 (*n* = 10), t2011 (*n* = 16), and t034 (*n* = 52). The results indicated a shift from MRSA *spa* types t2011 and t011 before the intervention to t034 after the decontamination measures (Fig. 6): *spa* types t011 (35.3% [6/17]) and t2011 (70.6% [12/17]) were found in all samples (human, pig, air, dust, and water) obtained before the decontamination measure. In contrast, *spa* type t034 was detected in 98.0% (50/51) of all isolates (pig, air, dust, and water) collected after the intervention. No obvious differences in the distribution of the *spa* types between the new and the old stables appeared.

The previously predominant MRSA *spa* type t2011 was only recovered in water (collected from a water line temporarily disused during the decontamination period in the old stable) (Fig. 1) after decontamination. Pigs had no contact with this line (see arrow).

Transmission of MRSA to humans in the farm environment. To assess whether MRSA strains colonizing the farm personnel might be transmitted from pigs, we analyzed the *spa* types of MRSA isolates from nose swabs of the humans tested in this report. All four persons with MRSA-positive samples had contact with pigs.

The *spa* type determined in staff 1 during the first monitoring (t011) had been displaced by t2011 before the decontamination. This *spa* type was still detectable at days 1 to 2 and after 1 month of acquiring the new pigs. However, after 4 months, this person had acquired a new strain with the *spa* type t034 and lost the old strain with *spa* type t2011 (Table 1).

Two more employees (temporary staff) carried the *spa* type t011 or t2011 before the decontamination procedure. Two months after the decontamination procedure, one of them had

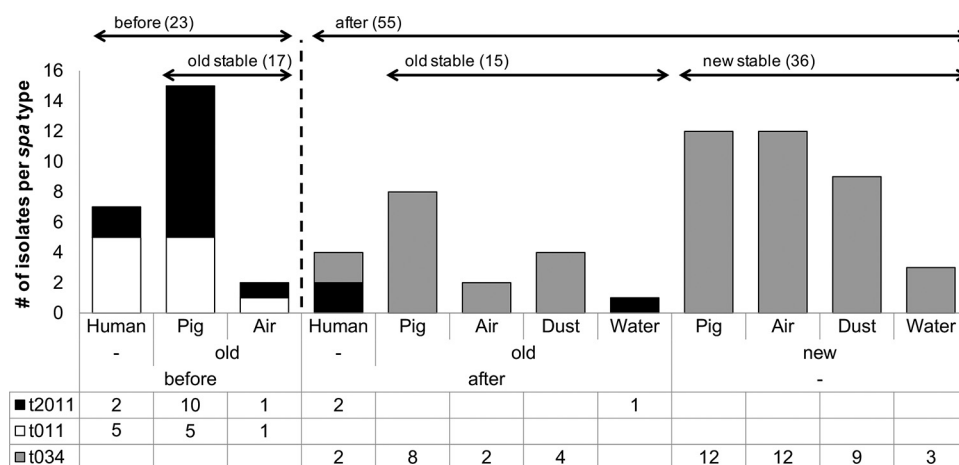


FIG 6 *spa* typing of MRSA isolates. Shown is the distribution of *spa* types relative to the time point of decontamination (before/after) of the old stable and in the new (–) stable.

lost colonization with *S. aureus*, whereas the other person was colonized with t034. The farmer was also colonized with LA-MRSA (t011) in two samples obtained before decontamination (one nasal sample and one wound sample) and turned out to be free of *S. aureus* after the decontamination procedure.

The results show a clear correlation between *spa* types in humans and *spa* types in pigs and environmental samples (Fig. 6 and Table 1). They further reveal a shift in colonizing MRSA strains observed in pigs and the environment (Fig. 6). However, in one person, this shift was delayed compared to that in the pigs and stable environment.

Prevalence of MRSA in companion animals. Companion animals are described as potential reservoirs of MRSA and ESBL-E (54–60). In our report, nasal swabs (one per animal and per time point) obtained from two dogs (eight samples) and two horses (eight samples) were MRSA negative. Of note, these animals had no access to the pig stables.

Dust samples in the stable environment. Native dust samples and dust swabs were collected; however, only native samples were used for further analysis. This was due to the fact that MRSA was identified in 18.5% (32/184) of native dust samples compared to only 10.9% (20/184) of dust swabs. In 10 cases, both methods yielded corresponding results; noncorresponding results might be due to sampling errors due to a nonhomogenous distribution of bacteria in the dust (61). Initially, MRSA detection in the dust samples in the old stable was reduced after decontamination

(Fig. 5). It was even lower in the new stable, where only a few compartments housed pigs and all empty compartments were free of MRSA.

Usage of antibiotics on the farm during the report period. At the sampling time point 9 months before the conversion, β -lactam antibiotics and tetracycline (doxycycline) were mostly administered to piglets and sows. In the months before the conversion, the farmer removed all animals with a weak health status; this led to a strong decrease in the use of antibiotics in the period leading up to the sampling time point 1 to 2 days before conversion and might have caused the decrease in ESBL-E encountered in these samples. After conversion, every new batch of incoming gilts and sows (staggered age groups of 160 to 200 days with weights of 90 to 100 kg) received prophylaxis with doxycycline for 10 days via oral fodder application. The selective pressure generated by the high use of tetracycline explains the high number of tetracycline-resistant strains. In fact, 100% of the MRSA isolates showed resistance to tetracycline, which is a characteristic of CC398 LA-MRSA. One year after decontamination, the farmer had abrogated the use of antibiotics because of a continuous absence of gastrointestinal disease.

DISCUSSION

Although LA-MRSA strains themselves are not a strong concern for pig health, they are indicators for a poor health status of the herd and frequent antibiotic treatments. Additionally, these bac-

TABLE 1 *spa* types from humans depending on the sampling time point

		<i>spa</i> type by time point of sampling ^a														
		Before decontamination						After decontamination								
		9 mo			1–2 days			1–2 days			1 mo			2 mo		
Human	Pig contact	t011	t034	t034	t011	t034	t034	t011	t034	t034	t011	t034	t034	t011	t034	t034
Farmer	Yes	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–
Staff 1	Yes	+	–	–	–	+	–	–	+	–	–	+	–	–	–	+
Staff 2	Yes	*	*	*	+	–	–	*	*	*	–	–	+	*	*	*
Staff 3	Yes	*	*	*	–	+	–	*	*	*	–	–	–	*	*	*
Farmer's wife	No	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–

^a +, positive for *spa* type; –, negative for *spa* type; *, not tested.

teria have a serious pathogenic potential for humans and therefore pose a risk to consumers and producers (62).

The present report describes the first attempt at total elimination (eradication) of MRSA and ESBL-E from a pig farm. So far, only one previous study reports eradication measures for MRSA on two horse farms; however, only short-term eradication of MRSA was achieved (30). In contrast, by the approach used in this study the original LA-MRSA strains (*spa* types t011 and t2011) were eliminated from both the environment and the pigs (Fig. 6). Additionally, ESBL-E isolates were completely absent after the disinfection procedure (Fig. 4 and 5). Thus, the decontamination measures performed in this report can be regarded as successful.

Nevertheless, after decontamination the original MRSA strains were replaced by a new LA-MRSA strain with *spa* type t034 (Fig. 6); the source of this new strain is unclear. On the one hand, it has been proposed that colonized human subjects represent a possible source of LA-MRSA for transmission to animals (63–65). This might be particularly true in breeding farms where personnel have close and very frequent contact with horses (30) or nursing sows and suckling piglets (66). However, the *spa* typing revealed that the new strain was not detectable in the farm personnel in the first 2 months after decontamination. On the other hand, introduction of only a few MRSA-positive animals into a pig stable with MRSA-negative pigs will constitute a reservoir for new colonization (67–70). *spa* repeats can be removed or duplicated by slipped-strand mutation. This way, one mutational event would be required to transform t011 to t034 and t011 to t2011, but two steps would be necessary to transform t2011 (found in the human specimen) to t034. Despite the relatedness of the *spa* types, we therefore hypothesize that due to the complete absence of t011 after the disinfection measures, it is very unlikely that the new t034 MRSA population originated from mutation of the old strain.

To exclude reintroduction of MRSA and ESBL-E after the decontamination, pigs and dust had been screened for MRSA and ESBL-E on the supplier farm. An MRSA- and ESBL-E-negative cohort was selected based on these examinations and transferred to the farm. However, the pigs were only transferred 3 months after microbiological analysis, offering the possible explanation that LA-MRSA might have been acquired during this period (71, 72). Thus, the conversion of the farm to a closed pig production system could have been a very effective means for prevention of MRSA recolonization (73, 74); however, the insufficient control of colonization of the incoming animals represents an important pitfall. A decline in ESBL-E colonization was also visible at the sampling just before decontamination and might be caused by the better health status and by the fact that farmer had stopped dispensing antibiotics.

MRSA is frequently present in environmental samples (Fig. 5) (33). In horse stables, MRSA has been isolated from 62% of stall surfaces, including walls, doors, water bowls, feed bowls, and hay nets (75). Additionally, LA-MRSA can be transmitted via air and is emitted via ventilation systems into the ambient air (33, 76, 77). This fact could explain how one air sample from a restricted and foil-separated area without pigs was positive. Otherwise, all compartments free of pigs were also free of MRSA, indicating that the disinfection measures had been effective, especially since MRSA can survive on dust for several months (33). Furthermore, dust samples were MRSA positive whenever the microorganisms were detected in the air (Fig. 5), an observation also made by Friese et al. (33). Interestingly, colonization of the two affected employees either ceased or switched to the new

LA-MRSA strain in the first months after decontamination (Table 1). This finding agrees with a previous report showing that humans working on pig farms carry MRSA of the same sequence type as the pigs (66, 78). Thus, our report demonstrates that in this case, the environmental predominance of an MRSA strain outcompeted the preexisting colonization of personnel with other MRSA strains. Considering the presence of the MRSA strain in the air and dust of the stable, this observation confirms earlier reports that indicate that MRSA detected in the nose swabs may well be acquired by breathing stable air (33, 79).

Our results show that the control of MRSA colonization can be achieved with basic but rather aggressive infection control measures (as in our case total decontamination and new construction of stables and culling of pigs, including decolonization of farm-workers and infection control measures). The conversion and disinfection procedures applied in this report are in line with those recommended (54, 80). The stable installations were constructed of metal and plastic. These surface types are considered to be relatively easily disinfected compared with wooden surfaces (75).

Because of the drastic and costly measures applied here, the question arises of whether only active screening and strict implementation of infection control protocols (30) would have been completely sufficient for elimination of LA-MRSA and ESBL-E. Friese et al. (33) conclude that very effective cleaning and disinfection of the stables, including all ventilation systems, before housing with new incoming pigs are necessary to avoid transmission of MRSA between subsequent fattening groups or groups of animals within breeding farms by contaminated premises. Similar to our approach, Friese et al. (33) performed dust sampling before and after replacement of animals and cleaning and disinfection of stables; however, MRSA was not eradicated.

For calves, an inverse association was found between MRSA carriage and farm hygiene (odds ratio [OR], 0.3): i.e., cleaning of stables before entrance of new calf populations to the farm. Disinfection was applied in less than 20% of the farms and was not negatively associated with MRSA carriage in calves (81). These results suggest that less severe methods may not have been successful.

In an interview with the farmer 1 year after decontamination, the farmer observed substantial benefits of the measures that had resulted in a strong decrease in the use of antibiotics due to the abandonment of the prophylaxis for incoming pigs. Furthermore, he reported that the incidence of diarrhea had dropped to zero, that the litters were more homogenous, that the scattering effect within a litter was not as high, and that the loss rate in piglets was below 2%. He assumed that a strong stable immunity within the herd had developed as the piglets born in the new system showed no health fluctuations. Nevertheless, the farmer was still participating in continuous and uniform monitoring programs that entail several diagnostic procedures.

In conclusion, we have shown here that eradication of resistant bacteria from a pig farm is costly but possible and conveys benefits. However, reintroduction of colonized new animals must be avoided by very intensive screening shortly before purchase and transfer.

ACKNOWLEDGMENTS

We thank the very supportive farmer and his family and employees for giving us the opportunity to carry out this study. We thank M. El-Jade for performing CTX-M PCR. We also thank K. Behringer for giving expert

advice and support regarding water sampling. We gratefully acknowledge T. Schulze Horsel for on-site support. We are indebted to P. Heinrich for active support during the total report period. This publication made use of the *spa* typing website (<http://www.spaserver.ridom.de/>) that was developed by Ridom GmbH and curated by SeqNet.org (<http://www.SeqNet.org/>).

This work was supported by the German-Dutch cooperation project "Safe Guard" (II-2-03 = 025). The project is financed via the INTERREG IV A-program Germany-Netherlands (ERDF) and cofinanced by the Ministry of Economic Affairs of North Rhine-Westphalia, the Lower Saxony State Chancellery, the Ministry of Economic Affairs of the Netherlands, and the Dutch Provinces Gelderland, Overijssel, Drenthe, Friesland, Noord-Brabant, Limburg, and Groningen (www.deutschland-nederland.eu).

REFERENCES

1. Beneke B, Klees S, Stührenberg B, Fetsch A, Kraushaar B, Tenhagen BA. 2011. Prevalence of methicillin-resistant *Staphylococcus aureus* in a fresh meat pork production chain. *J Food Prot* 74:126–129. <http://dx.doi.org/10.4315/0362-028X.JFP-10-250>.
2. Broens EM, Graat EA, van der Wolf PJ, van de Giessen AW, van Duikeren E, Wagenaar JA, van Nes A, Mevius DJ, de Jong MC. 2011. MRSA CC398 in the pig production chain. *Prev Vet Med* 98:182–189. <http://dx.doi.org/10.1016/j.prevetmed.2010.10.010>.
3. de Neeling AJ, van den Broek MJ, Spalburg EC, van Santen-Verheul MG, Dam-Deisz WD, Boshuizen HC, van de Giessen AW, van Duikeren E, Huijsdens XW. 2007. High prevalence of methicillin resistant *Staphylococcus aureus* in pigs. *Vet Microbiol* 122:366–372. <http://dx.doi.org/10.1016/j.vetmic.2007.01.027>.
4. Lassok B, Tenhagen BA. 2013. From pig to pork: methicillin-resistant *Staphylococcus aureus* in the pork production chain. *J Food Prot* 76:1095–1108. <http://dx.doi.org/10.4315/0362-028X.JFP-12-341>.
5. Geser N, Stephan R, Kuhnert P, Zbinden R, Kaeppli U, Cernela N, Haechler H. 2011. Fecal carriage of extended-spectrum β -lactamase-producing *Enterobacteriaceae* in swine and cattle at slaughter in Switzerland. *J Food Prot* 74:446–449. <http://dx.doi.org/10.4315/0362-028X.JFP-10-372>.
6. Blanc V, Mesa R, Saco M, Lavilla S, Prats G, Miró E, Navarro F, Cortés P, Llagostera M. 2006. ESBL- and plasmidic class C β -lactamase-producing *E. coli* strains isolated from poultry, pig and rabbit farms. *Vet Microbiol* 118:299–304. <http://dx.doi.org/10.1016/j.vetmic.2006.08.002>.
7. Tian GB, Wang HN, Zou LK, Tang JN, Zhao YW, Ye MY, Tang JY, Zhang Y, Zhang AY, Yang X, Xu CW, Fu YJ. 2009. Detection of CTX-M-15, CTX-M-22, and SHV-2 extended-spectrum β -lactamases (ESBLs) in *Escherichia coli* fecal-sample isolates from pig farms in China. *Foodborne Pathog Dis* 6:297–304. <http://dx.doi.org/10.1089/fpd.2008.0164>.
8. Hunter PA, Dawson S, French GL, Goossens H, Hawkey PM, Kuijper EJ, Nathwani D, Taylor DJ, Teale CJ, Warren RE, Wilcox MH, Woodford N, Wulf MW, Piddock LJ. 2010. Antimicrobial-resistant pathogens in animals and man: prescribing, practices and policies. *J Antimicrob Chemother* 65(Suppl 1):i3–i17. <http://dx.doi.org/10.1093/jac/dkp433>.
9. Hawkey PM, Jones AM. 2009. The changing epidemiology of resistance. *J Antimicrob Chemother* 64(Suppl 1):i3–i10. <http://dx.doi.org/10.1093/jac/dkp256>.
10. Li XZ, Mehrotra M, Ghimire S, Adewoye L. 2007. Beta-lactam resistance and beta-lactamases in bacteria of animal origin. *Vet Microbiol* 121:197–214. <http://dx.doi.org/10.1016/j.vetmic.2007.01.015>.
11. Cuny C, Nathaus R, Leyer F, Strommenger B, Altmann D, Witte W. 2009. Nasal colonization of humans with methicillin-resistant *Staphylococcus aureus* (MRSA) CC398 with and without exposure to pigs. *PLoS One* 4:e6800. <http://dx.doi.org/10.1371/journal.pone.0006800>.
12. Deiters C, Günnewig V, Friedrich AW, Mellmann A, Köck R. 2015. Are cases of methicillin-resistant *Staphylococcus aureus* clonal complex (CC) 398 among humans still livestock-associated? *Int J Med Microbiol* 305:110–113. <http://dx.doi.org/10.1016/j.ijmm.2014.11.007>.
13. van den Bogaard AE, Stobberingh EE. 2000. Epidemiology of resistance to antibiotics. Links between animals and humans. *Int J Antimicrob Agents* 14:327–335.
14. Marshall BM, Levy SB. 2011. Food animals and antimicrobials: impacts on human health. *Clin Microbiol Rev* 24:718–733. <http://dx.doi.org/10.1128/CMR.00002-11>.
15. Threlfall EJ, Ward LR, Frost JA, Willshaw GA. 2000. The emergence and spread of antibiotic resistance in food-borne bacteria. *Int J Food Microbiol* 62:1–5. [http://dx.doi.org/10.1016/S0168-1605\(00\)00351-2](http://dx.doi.org/10.1016/S0168-1605(00)00351-2).
16. Aarestrup FM, Hasman H, Agersø Y, Jensen LB, Harksen S, Svensmark B. 2006. First description of blaCTX-M-1-carrying *Escherichia coli* isolates in Danish primary food production. *J Antimicrob Chemother* 57:1258–1259. <http://dx.doi.org/10.1093/jac/dkl109>.
17. Liebana E, Batchelor M, Hopkins KL, Clifton-Hadley FA, Teale CJ, Foster A, Barker L, Threlfall EJ, Davies RH. 2006. Longitudinal farm study of extended-spectrum β -lactamase-mediated resistance. *J Clin Microbiol* 44:1630–1634. <http://dx.doi.org/10.1128/JCM.44.5.1630-1634.2006>.
18. de Boer E, Zwartkruis-Nahuis JT, Wit B, Huijsdens XW, de Neeling AJ, Bosch T, van Oosterom RA, Vila A, Heuvelink AE. 2009. Prevalence of methicillin-resistant *Staphylococcus aureus* in meat. *Int J Food Microbiol* 134:52–56. <http://dx.doi.org/10.1016/j.ijfoodmicro.2008.12.007>.
19. Wulf MW, Verduin CM, van Nes A, Huijsdens X, Voss A. 2012. Infection and colonization with methicillin resistant *Staphylococcus aureus* ST398 versus other MRSA in an area with a high density of pig farms. *Eur J Clin Microbiol Infect Dis* 31:61–65. <http://dx.doi.org/10.1007/s10096-011-1269-z>.
20. van Cleef BA, Verkade EJ, Wulf MW, Buiting AG, Voss A, Huijsdens XW, van Pelt W, Mulders MN, Kluytmans JA. 2010. Prevalence of livestock-associated MRSA in communities with high pig-densities in The Netherlands. *PLoS One* 5:e9385. <http://dx.doi.org/10.1371/journal.pone.0009385>.
21. Monaco M, Pedroni P, Sanchini A, Bonomini A, Indelicato A, Pantosti A. 2013. Livestock-associated methicillin-resistant *Staphylococcus aureus* responsible for human colonization and infection in an area of Italy with high density of pig farming. *BMC Infect Dis* 13:258. <http://dx.doi.org/10.1186/1471-2334-13-258>.
22. Aubry-Damon H, Grenet K, Sall-Ndiaye P, Che D, Cordeiro E, Bougnoux ME, Rigaud E, Le Strat Y, Lemanissier V, Armand-Lefevre L, Delzescaux D, Desenclos JC, Liénard M, Andremont A. 2004. Antimicrobial resistance in commensal flora of pig farmers. *Emerg Infect Dis* 10:873–879. <http://dx.doi.org/10.3201/eid1005.030735>.
23. Köck R, Schaumburg F, Mellmann A, Köksal M, Jurke A, Becker K, Friedrich AW. 2013. Livestock-associated methicillin-resistant *Staphylococcus aureus* (MRSA) as causes of human infection and colonization in Germany. *PLoS One* 8:e55040. <http://dx.doi.org/10.1371/journal.pone.0055040>.
24. Köck RB, Bischoff B, Cuny M, Eckmanns C, Fetsch T, Harmsen A, Goerge D, Oberheitmann T, Schwarz B, Selhorst S, Tenhagen T, Walther BA, Witte B, Ziebuhr W, Becker WK. 2014. The burden of zoonotic MRSA colonization and infection in Germany. *Berl Munch Tierarztl Wochenschr* 127:384–398.
25. Bisdorff B, Scholthöfer JL, Claußen K, Pulz M, Nowak D, Radon K. 2012. MRSA-ST398 in livestock farmers and neighbouring residents in a rural area in Germany. *Epidemiol Infect* 140:1800–1808. <http://dx.doi.org/10.1017/S0950268811002378>.
26. Johnson JR, Kuskowski MA, Smith K, O'Bryan TT, Tatini S. 2005. Antimicrobial-resistant and extraintestinal pathogenic *Escherichia coli* in retail foods. *J Infect Dis* 191:1040–1049. <http://dx.doi.org/10.1086/428451>.
27. Overdevest I, Willemsen I, Rijnsburger M, Eustace A, Xu L, Hawkey P, Heck M, Savelkoul P, Vandenbroucke-Grauls C, van der Zwaluw K, Huijsdens X, Kluytmans J. 2011. Extended-spectrum β -lactamase genes of *Escherichia coli* in chicken meat and humans, The Netherlands. *Emerg Infect Dis* 17:1216–1222. <http://dx.doi.org/10.3201/eid1707.110209>.
28. Jakobsen L, Spangholm DJ, Pedersen K, Jensen LB, Emborg HD, Agersø Y, Aarestrup FM, Hammerum AM, Frimodt-Møller N. 2010. Broiler chickens, broiler chicken meat, pigs and pork as sources of ExPEC related virulence genes and resistance in *Escherichia coli* isolates from community-dwelling humans and UTI patients. *Int J Food Microbiol* 142:264–272. <http://dx.doi.org/10.1016/j.ijfoodmicro.2010.06.025>.
29. Petersen B, Knura-Deszczka S, Pönsen-Schmidt E, Gymnich S. 2002. Computerised food safety monitoring in animal production. *Livest Prod Sci* 76:207–213. [http://dx.doi.org/10.1016/S0301-6226\(02\)00120-3](http://dx.doi.org/10.1016/S0301-6226(02)00120-3).
30. Weese JS, Rousseau J. 2005. Attempted eradication of methicillin-resistant *Staphylococcus aureus* colonisation in horses on two farms. *Equine Vet J* 37:510–514.
31. Williams AP, Avery LM, Killham K, Jones DL. 2005. Persistence of *Escherichia coli* O157 on farm surfaces under different environmental con-

- ditions. *J Appl Microbiol* 98:1075–1083. <http://dx.doi.org/10.1111/j.1365-2672.2004.02530.x>.
32. EFSA. 2009. Analysis of the baseline survey on the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in holdings with breeding pigs, in the EU, 2008. *EFSA J* 7:1–82.
 33. Friese A, Schulz J, Hoehle L, Fetsch A, Tenhagen BA, Hartung J, Roesler U. 2012. Occurrence of MRSA in air and housing environment of pig barns. *Vet Microbiol* 158:129–135. <http://dx.doi.org/10.1016/j.vetmic.2012.01.019>.
 34. Hu YY, Cai JC, Zhou HW, Chi D, Zhang XF, Chen WL, Zhang R, Chen GX. 2013. Molecular typing of CTX-M-producing *Escherichia coli* isolates from environmental water, swine feces, specimens from healthy humans, and human patients. *Appl Environ Microbiol* 79:5988–5996. <http://dx.doi.org/10.1128/AEM.01740-13>.
 35. Martinez JL. 2009. The role of natural environments in the evolution of resistance traits in pathogenic bacteria. *Proc Biol Sci* 276:2521–2530. <http://dx.doi.org/10.1098/rspb.2009.0320>.
 36. Martinez JL. 2008. Antibiotics and antibiotic resistance genes in natural environments. *Science* 321:365–367. <http://dx.doi.org/10.1126/science.1159483>.
 37. Blake DP, Hillman K, Fenlon DR, Low JC. 2003. Transfer of antibiotic resistance between commensal and pathogenic members of the *Enterobacteriaceae* under ileal conditions. *J Appl Microbiol* 95:428–436. <http://dx.doi.org/10.1046/j.1365-2672.2003.01988.x>.
 38. von-Borell E, Bockisch F-J, Büscher W, Hoy S, Krieter J, Müllerf C, Parvizi N, Richter T, Rudovsky A, Sundrum A, Van den Weghe H. 2001. Critical control points for on-farm assessment of pig housing. *Livest Prod Sci* 72:177–184. [http://dx.doi.org/10.1016/S0301-6226\(01\)00278-0](http://dx.doi.org/10.1016/S0301-6226(01)00278-0).
 39. Wulf MW, Sørum M, van Nes A, Skov R, Melchers WJ, Klaassen CH, Voss A. 2008. Prevalence of methicillin-resistant *Staphylococcus aureus* among veterinarians: an international study. *Clin Microbiol Infect* 14:29–34. <http://dx.doi.org/10.1111/j.1469-0691.2007.01873.x>.
 40. Denis O, Suetens C, Hallin M, Catry B, Ramboer I, Dispas M, Willems G, Gordts B, Butaye P, Struelens MJ. 2009. Methicillin-resistant *Staphylococcus aureus* ST398 in swine farm personnel, Belgium. *Emerg Infect Dis* 15:1098–1101. <http://dx.doi.org/10.3201/eid1507.080652>.
 41. Mannion C, Leonard FC, Lynch PB, Egan J. 2007. Efficacy of cleaning and disinfection on pig farms in Ireland. *Vet Rec* 161:371–375. <http://dx.doi.org/10.1136/vr.161.11.371>.
 42. Van den Eede A, Hermans K, Van den Abeele A, Flore K, Dewulf J, Vanderhaeghen W, Nemeghaire S, Butaye P, Gasthuys F, Haesebrouck F, Martens A. 2013. The nasal vestibulum is the optimal sampling site for MRSA screening in hospitalised horses. *Vet J* 197:415–419. <http://dx.doi.org/10.1016/j.tvjl.2013.01.031>.
 43. Wedley AL, Dawson S, Maddox TW, Coyne KP, Pinchbeck GL, Clegg P, Jamrozzy D, Fielder MD, Donovan D, Nuttall T, Williams NJ. 2014. Carriage of *Staphylococcus* species in the veterinary visiting dog population in mainland UK: molecular characterisation of resistance and virulence. *Vet Microbiol* 170:81–88. <http://dx.doi.org/10.1016/j.vetmic.2014.01.015>.
 44. Feller W. 1948. On the Kolmogorov-Smirnov limit theorems for empirical distributions. *Ann Math Stat* 19:177–189. <http://dx.doi.org/10.1214/aoms/1177730243>.
 45. TrinkwV. 2001. Verordnung über die Qualität von Wasser für den menschlichen Gebrauch (Trinkwasserverordnung-TrinkwV 2001)neugefasst B. v. 02.08.2013 BGBl. I S. 2977; zuletzt geändert durch Artikel 4 G. v. 07.08.2013 BGBl. I S. 3154; Geltung ab 01.01.2003FNA: 2126-13-1; 2 Verwaltung 21 Besondere Verwaltungszweige der inneren Verwaltung 212 Gesundheitswesen 2126.
 46. Schulz J, Hartung J. 2009. Detection of MRSA in pig house air by impingement followed by membrane filtration. *Gefahrstoffe-Reinhaltung Luft* 69:348–352.
 47. EUCAST ECcAST. 2013. Clinical breakpoints—Bacteria v3.1. EUCAST, Växjö, Sweden. <http://www.eucast.org>.
 48. Harmsen D, Claus H, Witte W, Rothgänger J, Turnwald D, Vogel U. 2003. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for *spa* repeat determination and database management. *J Clin Microbiol* 41:5442–5448. <http://dx.doi.org/10.1128/JCM.41.12.5442-5448.2003>.
 49. Vossenkühl B, Brandt J, Fetsch A, Käsbohrer A, Kraushaar B, Alt K, Tenhagen BA. 2014. Comparison of *spa* types, SCCmec types and antimicrobial resistance profiles of MRSA isolated from turkeys at farm, slaughter and from retail meat indicates transmission along the production chain. *PLoS One* 9:e96308. <http://dx.doi.org/10.1371/journal.pone.0096308>.
 50. Dierikx CM, van der Goot JA, Smith HE, Kant A, Mevius DJ. 2013. Presence of ESBL/AmpC-producing *Escherichia coli* in the broiler production pyramid: a descriptive study. *PLoS One* 8:e79005. <http://dx.doi.org/10.1371/journal.pone.0079005>.
 51. Laube H, Friese A, von Salviati C, Guerra B, Rösler U. 2014. Transmission of ESBL/AmpC-producing *Escherichia coli* from broiler chicken farms to surrounding areas. *Vet Microbiol* 172:519–527. <http://dx.doi.org/10.1016/j.vetmic.2014.06.008>.
 52. Wagenaar JA, Yue H, Pritchard J, Broekhuizen-Stins M, Huijsdens X, Mevius DJ, Bosch T, Van Duinkerken E. 2009. Unexpected sequence types in livestock associated methicillin-resistant *Staphylococcus aureus* (MRSA): MRSA ST9 and a single locus variant of ST9 in pig farming in China. *Vet Microbiol* 139:405–409. <http://dx.doi.org/10.1016/j.vetmic.2009.06.014>.
 53. von Salviati C, Laube H, Guerra B, Roesler U, Friese A. 2015. Emission of ESBL/AmpC-producing *Escherichia coli* from pig fattening farms to surrounding areas. *Vet Microbiol* 175:77–84. <http://dx.doi.org/10.1016/j.vetmic.2014.10.010>.
 54. Catry B, Van Duinkerken E, Pomba MC, Greko C, Moreno MA, Pyörälä S, Ruzauskas M, Sanders P, Threlfall EJ, Ungemach F, Törneke K, Munoz-Madero C, Torren-Edo J, Scientific Advisory Group on Antimicrobials (SAGAM). 2010. Reflection paper on MRSA in food-producing and companion animals: epidemiology and control options for human and animal health. *Epidemiol Infect* 138:626–644. <http://dx.doi.org/10.1017/S0950268810000014>.
 55. Dierikx CM, van Duinkerken E, Schoormans AH, van Essen-Zandbergen A, Veldman K, Kant A, Huijsdens XW, van der Zwaluw K, Wagenaar JA, Mevius DJ. 2012. Occurrence and characteristics of extended-spectrum- β -lactamase- and AmpC-producing clinical isolates derived from companion animals and horses. *J Antimicrob Chemother* 67:1368–1374. <http://dx.doi.org/10.1093/jac/dks049>.
 56. Loeffler A, Lloyd DH. 2010. Companion animals: a reservoir for methicillin-resistant *Staphylococcus aureus* in the community? *Epidemiol Infect* 138:595–605. <http://dx.doi.org/10.1017/S0950268809991476>.
 57. Petinaki E, Spiliopoulou I. 2012. Methicillin-resistant *Staphylococcus aureus* among companion and food-chain animals: impact of human contacts. *Clin Microbiol Infect* 18:626–634. <http://dx.doi.org/10.1111/j.1469-0691.2012.03881.x>.
 58. Ewers C, Grobbel M, Stamm I, Kopp PA, Diehl I, Semmler T, Fruth A, Beutlich J, Guerra B, Wieler LH, Guenther S. 2010. Emergence of human pandemic O25:H4-ST131 CTX-M-15 extended-spectrum-beta-lactamase-producing *Escherichia coli* among companion animals. *J Antimicrob Chemother* 65:651–660. <http://dx.doi.org/10.1093/jac/dkq004>.
 59. Ewers C, Bethe A, Semmler T, Guenther S, Wieler LH. 2012. Extended-spectrum β -lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: a global perspective. *Clin Microbiol Infect* 18:646–655. <http://dx.doi.org/10.1111/j.1469-0691.2012.03850.x>.
 60. Wieler LH, Ewers C, Guenther S, Walther B, Lübke-Becker A. 2011. Methicillin-resistant staphylococci (MRS) and extended-spectrum beta-lactamases (ESBL)-producing *Enterobacteriaceae* in companion animals: nosocomial infections as one reason for the rising prevalence of these potential zoonotic pathogens in clinical samples. *Int J Med Microbiol* 301:635–641. <http://dx.doi.org/10.1016/j.ijmm.2011.09.009>.
 61. Lidwell OM, Lowbury EJ. 1950. The survival of bacteria in dust. I. The distribution of bacteria in floor dust. *J Hyg (Lond)* 48:6–20.
 62. Ballhausen B, Jung P, Kriegeskorte A, Makgotlho PE, Ruffing U, von Müller L, Köck R, Peters G, Herrmann M, Ziebuhr W, Becker K, Bischoff M. 2014. LA-MRSA CC398 differ from classical community acquired-MRSA and hospital acquired-MRSA lineages: functional analysis of infection and colonization processes. *Int J Med Microbiol* 304:777–786. <http://dx.doi.org/10.1016/j.ijmm.2014.06.006>.
 63. Seguin JC, Walker RD, Caron JP, Kloos WE, George CG, Hollis RJ, Jones RN, Pfaller MA. 1999. Methicillin-resistant *Staphylococcus aureus* outbreak in a veterinary teaching hospital: potential human-to-animal transmission. *J Clin Microbiol* 37:1459–1463.
 64. Weese JS, Archambault M, Willey BM, Hearn P, Kreiswirth BN, Said-Salim B, McGeer A, Likhoshvay Y, Prescott JF, Low DE. 2005. Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel, 2000–2002. *Emerg Infect Dis* 11:430–435. <http://dx.doi.org/10.3201/eid1103.040481>.

65. Weese JS. 2010. Methicillin-resistant *Staphylococcus aureus* in animals. ILAR J 51:233–244. <http://dx.doi.org/10.1093/ilar.51.3.233>.
66. van den Broek IV, van Cleef BA, Haenen A, Broens EM, van der Wolf PJ, van den Broek MJ, Huijsdens XW, Kluytmans JA, van de Giessen AW, Tiemersma EW. 2009. Methicillin-resistant *Staphylococcus aureus* in people living and working in pig farms. Epidemiol Infect 137:700–708. <http://dx.doi.org/10.1017/S0950268808001507>.
67. Broens EM, Graat EA, van de Giessen AW, Broekhuizen-Stins MJ, de Jong MC. 2012. Quantification of transmission of livestock-associated methicillin resistant *Staphylococcus aureus* in pigs. Vet Microbiol 155:381–388. <http://dx.doi.org/10.1016/j.vetmic.2011.09.010>.
68. van Duijkeren E, Ikawaty R, Broekhuizen-Stins MJ, Jansen MD, Spalburg EC, de Neeling AJ, Allaart JG, van Nes A, Wagenaar JA, Fluit AC. 2008. Transmission of methicillin-resistant *Staphylococcus aureus* strains between different kinds of pig farms. Vet Microbiol 126:383–389. <http://dx.doi.org/10.1016/j.vetmic.2007.07.021>.
69. Espinosa-Gongora C, Broens EM, Moodley A, Nielsen JP, Guardabassi L. 2012. Transmission of MRSA CC398 strains between pig farms related by trade of animals. Vet Rec 170:564. <http://dx.doi.org/10.1136/vr.100704>.
70. Crombé F, Vanderhaeghen W, Dewulf J, Hermans K, Haesebrouck F, Butaye P. 2012. Colonization and transmission of methicillin-resistant *Staphylococcus aureus* ST398 in nursery piglets. Appl Environ Microbiol 78:1631–1634. <http://dx.doi.org/10.1128/AEM.07356-11>.
71. Ciccolini M, Dahl J, Chase-Topping ME, Woolhouse ME. 2012. Disease transmission on fragmented contact networks: livestock-associated methicillin-resistant *Staphylococcus aureus* in the Danish pig-industry. Epidemics 4:171–178. <http://dx.doi.org/10.1016/j.epidem.2012.09.001>.
72. Broens EM, Graat EA, Van der Wolf PJ, Van de Giessen AW, De Jong MC. 2011. Transmission of methicillin resistant *Staphylococcus aureus* among pigs during transportation from farm to abattoir. Vet J 189:302–305. <http://dx.doi.org/10.1016/j.tvjl.2010.08.003>.
73. Vanderhaeghen W, Hermans K, Haesebrouck F, Butaye P. 2010. Methicillin-resistant *Staphylococcus aureus* (MRSA) in food production animals. Epidemiol Infect 138:606–625. <http://dx.doi.org/10.1017/S0950268809991567>.
74. Crombé F, Willems G, Dispas M, Hallin M, Denis O, Suetens C, Gordts B, Struelens M, Butaye P. 2012. Prevalence and antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* among pigs in Belgium. Microb Drug Resist 18:125–131. <http://dx.doi.org/10.1089/mdr.2011.0138>.
75. Weese JS, DaCosta T, Button L, Goth K, Ethier M, Boehnke K. 2004. Isolation of methicillin-resistant *Staphylococcus aureus* from the environment in a veterinary teaching hospital. J Vet Intern Med 18:468–470. <http://dx.doi.org/10.1111/j.1939-1676.2004.tb02568.x>.
76. Gibbs SG, Green CF, Tarwater PM, Scarpino PV. 2004. Airborne antibiotic resistant and nonresistant bacteria and fungi recovered from two swine herd confined animal feeding operations. J Occup Environ Hyg 1:699–706. <http://dx.doi.org/10.1080/15459620490515824>.
77. Schulz J, Frieze A, Klees S, Tenhagen BA, Fetsch A, Rösler U, Hartung J. 2012. Longitudinal study of the contamination of air and of soil surfaces in the vicinity of pig barns by livestock-associated methicillin-resistant *Staphylococcus aureus*. Appl Environ Microbiol 78:5666–5671. <http://dx.doi.org/10.1128/AEM.00550-12>.
78. Cuny C, Stanek C, Witte W. 2009. MRSA aus Sicht des RKI: Nachweise bei Menschen und anderen Tieren-eine kommende Zoonose? Tierarztl Wochenschr 116:284–290.
79. Masclaux FG, Sakwinska O, Charrière N, Semaani E, Oppliger A. 2013. Concentration of airborne *Staphylococcus aureus* (MRSA and MSSA), total bacteria, and endotoxins in pig farms. Ann Occup Hyg 57:550–557. <http://dx.doi.org/10.1093/annhyg/mes098>.
80. Sommer H, Greuel E, Müller W. 1976. Tierhygiene, Gesunderhaltung von Rindern und Schweinen. Eugen Ulmer GmbH and Co., Stuttgart, Germany.
81. Graveland H, Wagenaar JA, Heesterbeek H, Mevius D, van Duijkeren E, Heederik D. 2010. Methicillin resistant *Staphylococcus aureus* ST398 in veal calf farming: human MRSA carriage related with animal antimicrobial usage and farm hygiene. PLoS One 5:e10990. <http://dx.doi.org/10.1371/journal.pone.0010990>.